Proteinaceous macromolecules, or fragments thereof, derived from the surface of T cells, and proteinaceous macromolecules which have immunologically similar counterparts present on the surface of T cells or activated T cells, which are present in a body fluid and not associated with the surface of a T cell shall be herein referred to as soluble T cell surface molecules. These macromolecules can be either glycosylated or non-glycosylated and may be soluble by themselves or considered soluble by virtue of their association with other soluble macromolecules. Furthermore, macromolecules which are present in a fluid and are not bound to or associated with the surface of a cell are referred to as soluble macromolecules.

5.1. MONITORING THE EFFECT OF A THERAPEUTIC TREATMENT

The present invention provides a method for monitoring the effect of a therapeutic treatment on a subject who has undergone the therapeutic treatment. This method comprises measuring at suitable time intervals the amount of a soluble molecule or soluble fragment thereof which comprises, or is immunologically related to, a T cell growth factor receptor or T cell differentiation antigen. Any change or absence of change in the amount of the soluble molecule can be identified and correlated with the effect of the therapeutic treatment on the subject. In a specific embodiment of the invention, soluble molecules immunologically related to the interleukin-2 receptor (IL2R) can be measured in the serum of patients by a sandwich enzyme immunoassay (for an example, see Section 15, infra), in order to evaluate the therapeutic efficacy of, for example, administration of immunomodulators such as alpha-interferon, Cyclosporin A, and monoclonal antibody OKT3. In another embodiment, soluble molecules related to the interleukin-1 receptor can be measured.

The therapeutic treatments which may be evaluated according to the present invention include but are not limited to radiotherapy, drug administration, immunosuppressive or immunoenhancive regimens, etc. The immunosuppressant regimens include, but are not limited to administration of drugs such as Cyclosporin A, chlorambucil, cyclophosphamide, or azathioprine, and anti-T cell antibody such as anti-T3 monoclonal antibody and anti-thymocyte globulin, etc. The immunoenhancive regimens include, but are not limited to administration of interleukin-1, interleukin-2, and other T cell growth factors.

5.2. DETECTING AND/OR STAGING A DISEASE IN A SUBJECT

In another embodiment of the present invention, measurement of a soluble molecule which comprises, or is immunologically related to, a T cell growth factor receptor or T cell differentiation antigen can be used to detect and/or stage a disease or disorder in a subject. The measured amount of the soluble molecule is compared to standard amounts which are established to be normally present in the body fluid of subjects with various degrees of the disease or disorder. An amount present in the body fluid of the subject which is similar to a standard amount, established to be normally present in the body fluid of the subject during a specific stage of the disease or disorder, is indicative of the stage of the disease in the subject. Diseases or disorders which may be detected and/or staged in a subject according to the present

invention include but are not limited to those listed in Table II, infra.

TABLE II

DISEASES AND DISORDERS WHICH MAY BE DETECTED AND/OR STAGED IN A SUBJECT ACCORDING TO THE PRESENT INVENTION
I. Infectious Diseases
Induced by virus:
Herpesvirus
Cytomegalovirus
Epstein-Barr Virus
HTLV-I
HTLV-III/LAV/HIV (AIDS)
II. Cancer
T cell leukemia
HTLV-I-associated adult T cell leukemia
T cell lymphoma
Burkitt's lymphoma
Hairy cell leukemia
Sezary syndrome
Hodgkin's disease
Chronic lymphocytic leukemia
Non-Hodgkin's lymphoma
B-cell acute lymphoblastic leukemia
Solid tumors
III. Autoimmune Diseases
Rheumatoid arthritis
Diabetes
Multiple sclerosis
Systemic lupus erythematosis
IV. Organ Allograft Rejection

In specific embodiments of this aspect of the invention, measurements of plasma or serum levels of the IL2R or related molecules can be used in the detection of disease, or to determine disease stage and assign risk. For example, patients with lymphatic diseases and cancer such as non-Hodgkin's lymphoma or B cell acute lymphoblastic leukemia, or adult T cell leukemia can be monitored by measuring serum levels of soluble IL2R; elevated serum IL2R correlates directly with severity of the disease condition and indicates a poor response to therapy as well as a poor prognosis. In another example, the response of patients with non-lymphatic cancers to therapy with IL-2 can be monitored; in this case elevated serum levels of soluble IL2R indicates a positive response to IL-2 therapy. Responses to viral infections can also be monitored by measuring soluble IL2R levels in a patient. For example, patients infected with herpes virus or an AIDS virus present elevated serum levels of soluble IL2R. In another embodiment, plasma or serum IL2R levels can be measured in transplant patients; elevated serum levels of soluble IL2R is a diagnostic indication of allograft rejection. In another specific embodiment, T cell CD8-like molecules may be measured; detection of increased levels of soluble CD8 is associated with various diseases and disorders such as rheumatoid arthritis. Detection of elevated levels of a CD8-like antigen can indicate the involvement of significant numbers of suppressor/cytotoxic T cells with a specific pathological event, distinct from immune activation as measured by a rise in cell-free IL2R.

5.3. DIFFERENTIAL DIAGNOSIS OF A PHYSIOLOGICAL CONDITION

In another embodiment of the invention, the measurement of soluble T cell growth factor receptors, T cell surface antigens, or immunologically related molecules can be used to differentially diagnose in a subject a particular physiological condition as distinct from among two or more physiological conditions. To this end, the measured amount of the soluble molecule is compared with the amount of the soluble molecule normally present in a body fluid of a subject with one of the suspected physiological conditions. A measured amount of the soluble molecule similar to to the amount normally present in a subject with one of the physiological conditions, and not normally present in a subject with one or more of the other physiological conditions, is indicative of the physiological condition of the subject.

In a specific embodiment of this aspect of the invention, measurement of soluble molecules can be used in the differential diagnosis of renal allograft rejection, especially in distinguishing Cyclosporin A nephrotoxicity. In a particular embodiment of this aspect of the invention, the soluble molecules can be IL2R or related molecules. Similar differential diagnosis of allograft rejection using the methods of the invention can be applied to other organ allografts, including but not limited to liver, heart, and pancreas.

In another specific embodiment measurements of serum CD8 levels may be used in the differential diagnosis of rheumatoid arthritis, as distinguished from other joint diseases.

In yet another specific embodiment of the invention, lymphocytic leukemia may be differentially diagnosed, as distinguished from other leukemia, by measurement and detection of IL2R.

5.4. SOLUBLE T CELL GROWTH FACTOR RECEPTORS, T CELL DIFFERENTIATION ANTIGENS, AND RELATED MOLECULES

Any T cell surface molecule or immunologically related molecule which is present in soluble form in the body fluid at levels which correlate with a disease condition or disorder, or a stage thereof, may be used in the practice of the present invention. T cell surface markers which may potentially be used include but are not limited to those listed in Table I, supra.

Several of the markers listed in Table I, supra, are already known to exist in soluble form. CD2, a receptor for sheep red blood cells, has been detected at higher levels in the sera of certain cancer patients than in normal control patients (Falcao, R.P., et al., 1984, Clin. Lab. Immunol 13: 141-143, Oh, S.-K., et al., 1985, Scand. J. Immunol. 22: 51-60). Leu 2 (OKT8), a surface marker found on suppressor/cytotoxic T cells which may be involved in cellular recognition, has been reported at highly elevated levels in the serum of patients with T cell leukemia (Fujimoto, J., et al., 1983, J. Exp. Med. 159:752-766). Miller et al. (1982, N. Engl. J. Med. 306:517-520) reported the release of Leu 1 into the serum following monoclonal antibody treatment. However, Leu 1 antigen was not detectable in the serum of normal or leukemic patients who had not received antibody therapy.

Other T cell surface molecules whose soluble forms may be measured in accordance with the present invention include but are not limited to T cell growth factor receptors, e.g. interleukin-2 receptor and interleukin-1 receptor. In specific embodiments, serum IL2R measurements can be used to predict therapeutic outcomes and monitor the immune status of patients with cancer, immunodeficiencies, autoimmune diseases, or allograft rejection.

5.4.1. ASSAYS FOR MEASUREMENT

Any procedures known in the art for the measurement of soluble molecules can be used in the practice of the instant invention. Such procedures include but are not limited to competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, and immunoelectrophoresis assays, to name but a few.

In a preferred embodiment, a sandwich enzyme immunoassay can be used. One description of such an embodiment follows: A monoclonal antibody (capture antibody, mAb 1) directed against the soluble antigen is adsorbed onto a solid substratum. The soluble antigen present in the sample binds to the antibody, and unreacted sample components are removed by washing. An enzyme-conjugated monoclonal antibody (detection antobody, mAb 2) directed against a second epitope of the antigen binds to the antigen captured by mAb 1 and completes the sandwich. After removal of unbound mAb 2 by washing, a substrate solution is added to the wells. A colored product is formed in proportion to the amount of antigen present in the sample. The reaction is terminated by addition of stop solution and absorbance is measured spectrophotometrically. A standard curve is prepared from known concentrations of the soluble antigen, from which unknown sample values can be determined. In particular embodiments, such an assay may be used to determine soluble IL2R levels or soluble T cell antigen levels. In a preferred embodiment for the measurement of IL2R levels, anti-IL2R mAbs 2R12 and 7G7 can be used as the capture and detection antibodies, respectively, in a sandwich immunoassay (such as the CELLFREE™ assay described in Section 15 infra). In a preferred embodiment for the measurement of CD8

antigen levels, anti-CD8 mAbs 4C9 and 5F4 can be used as the capture and detection antibodies, respectively, in a sandwich enzyme immunoassay (such as described in Section 17, infra).

6. SOLUBLE IL2R DETECTION IN PATIENTS

The examples described in Sections 7 through 15 infra demonstrate the detection of soluble or cell free IL2R in patients and the utility of such detection for staging various diseases or conditions. The results indicate that (a) serum IL2R levels are elevated in patients with active lymphatic diseases such as leukemia and lymphoma. In such patients, serum IL2R levels bear a direct relationship with severity of disease and poor prognosis. (b) Serum IL2R levels are generally not elevated in patients with non-lymphatic cancers; however, IL-2 patients receiving IL-2 therapy who are responding to such therapy demonstrate elevated levels of serum IL2R. (c) Serum IL2R levels are elevated in transplant patients who reject allografts; however, serum IL2R levels are not elevated in patients who experience toxicity caused by immunosuppressive drugs used in transplant patients but do not demonstrate true allograft rejection.

The procedures used in these examples are described in the subsections below.

6.1. MONOCLONAL ANTIBODIES

Monoclonal antibodies directed against the IL2R were produced as previously described (Uchiyama, T., et al., 1981, J. Immunol. 126(4):1393-1397; Rubin, L.A., et al., 1985, Hybridoma 4:91-102; Jung, L.K.L., et al., 1984, J. Exp. Med. 160:1957). Additionally, monoclonal antibodies directed against IL2R may be purchased commercially (Becton-Dickenson, California; Coulter Diagnostic, Florida). Although the published monoclonal antibodies (Uchiyama, supra; Rubin, supra; Jung, supra) each recognize IL2R, each of these monoclonal antibodies recognizes a different epitope. Monoclonal antibodies were purified according to established standard methods (Cortheir, G., et al., 1984, J. Immuno. Method 66:75-79). Horseradish peroxidase conjugation was done according to published procedures (Wilson, M.B. and Nakane, P.K., 1978, "Recent Developments in the Periodate Method of Conjugating Horseradish Peroxide (HRPO) to Antibodies", in Immunofluorescence and Related Staining Techniques, Knapp, W., K. Holubar, and G. Wick, eds., Elsevier/North-Holland Biomedical Press, pp. 215-224).

6.2. SOLUBLE IL2R ASSAY

In the examples described in Sections 7 through 14, either the procedure described below or the CELLFREE™ Interleukin-2 Receptor Test Kit (T-Cell Sciences, Inc., Cambridge, MA) described in Section 15 infra was used to measure the amount of soluble IL2R in clinical samples. In each of these assay procedures, two monoclonal antibodies, each recognizing a different epitope on the target antigen, was used.

6.2.1. PROCEDURE

Soluble IL2R was detected in samples using the procedure outlined below:

(a) Polystyrene microliter wells (Flow Laboratory) were coated overnight at room temperature with 100 ul of an anti-IL2R murine monoclonal antibody (2 ug/ml) in phosphate buffered saline (PBS).
(b) Coating solution was discarded and wells were blocked for 1-2 hours at room temperature with 300 ul of 1% bovine serum albumin (BSA) in Tris-buffered saline containing 25 mM Tris pH 7.4 in 0.05% of Tween 20 and 0.15 M sodium chloride (Tris-Tween buffer).
(c) Wells were washed 3 times with Tris-Tween buffer.
(d) 50 ul sample was added per well, followed by 100 ul diluent containing fetal calf serum (FCS) in Tris-Tween buffer. Wells were incubated 2 hours at 37°C.
(e) Wells were washed 3 times with Tris-Tween buffer.
(f) 100 ul of horseradish peroxidase (Sigma Chemical Co.) conjugated anti-IL2R monoclonal antibody in PBS containing 50% FCS was added per well. Wells were incubated 2 hours at 37°C.
(g) Wells were washed 4 times with Tris-Tween buffer.
(h) 100 ul of 0.2% o-phenylenediamine (OPD) and 0.015% of H_2O_2 in 17 mM citric acid, 65 mM dibasic sodium phosphate (citrate-phosphate buffer) was added per well. Plates were incubated for 30 minutes at room temperature.
(i) 50 ul of 2 N H_2SO_4 was added to each well and the absorbance of each well was measured at 490 nm in a microtiter plate reader (Dynatech MR600, Dynatech Corp., Alexandria, VA.).